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Introduction

There is abundant evidence suggesting that catecholamines administered centrally can regulate adrenocorticotropin (ACTH) and vasopressin (AVP) release (2,4,34,40). Because there is a heavy catecholaminergic projection from the brainstem innervating the paraventricular nucleus (PVN) of the hypothalamus (37,39), the nuclear region that regulates AVP and ACTH release from the pituitary (16,18,20), it has been suggested that catecholaminergic regulation of the ACTH and AVP may occur directly at this site (2,4). The catecholaminergic cell groups in the brainstem that project to the paraventricular nucleus are located in regions shown to be involved in the baroreflexes and the regulation of arterial blood pressure and heart rate during hemorrhage (9,10,17,31,37,39,41). Recently, type II glucocorticoid receptors have been found on catecholaminergic cell groups in the brainstem (22). Coupled with the findings that ACTH and AVP immunostaining (7,8,27) and mRNA levels (6,19) are altered after adenalectomy, these data suggest that the responses of plasma ACTH and AVP and the recovery of arterial blood pressure and heart rate after hemorrhage and the baroreflex, may be influenced by glucocorticoids which may work though these catecholaminergic cell groups.

The goal of this past year was to 1) continue microinjection studies and determine a dose-response relationship between ACTH release and norepinephrine microinjected directly into the PVN, 2) to begin determining if microinjections of norepinephrine into the PVN can influence AVP release, 3) to determine if the loss and replacement of glucocorticoids effects baroreflex function, 4) and to determine if the recovery of arterial pressure and heart rate and the responses of plasma ACTH, AVP, oxytocin, norepinephrine, osmolality, Na^+, K^+, protein and gases to hemorrhage are affected by the loss of glucocorticoids. Also, since our recent evidence strongly suggested that the nutritional state of the animal affects the ability of an adrenalectomized animal to recover arterial pressure after hemorrhage, and that glucocorticoids are critical for the mobilization of body fuels during stress, possibly through brainstem catecholamine centers (25) and the PVN (28), we have also characterized the

responses of the above variables in fed and fasted states.

Material and Methods

Microinjections into the PVN: Male Sprague-Dawley rats (250-350) were anesthetized with pentobarbital sodium (45mg/kg,ip) and cannulae were placed in the femoral artery and vein (PE-50) for measurement of arterial pressure, heart rate and for blood withdrawal. The rats were placed in a stereotaxic device, the skin and muscle of the cranium was retracted, and a burr hole was drilled at the coordinates described below. Each rat was allowed 1 hour to stabilize before any further manipulation. Arterial blood pressure and heart rate were monitored continuously throughout each experiment. A glass micropipette (approx. 50um OD), filled with freshly prepared norepinephrine (10^{-9} to 10^{-2}M) in artificial CSF, was lowered into the PVN. Ten minutes later, 50nl was injected over two minutes. Injections were unilateral. Blood samples (1ml) were taken before and five minutes after the completion of the injection. Some animals were microinjected with artificial CSF.

Plasma ACTH or AVP were determined from the samples. Plasma ACTH was measured by radioimmunoassay (RIA) from glass-extracted plasma as described previously (13). The lowest detectable concentration was 10 pg/ml: intra- and interassay variations were 7 and 9.5% respectively. Vasopressin was assayed by RIA from bentonite-extracted plasma (42). The lowest detectable concentration was 0.3pg/ml; intra- and interassay variations were 9 and 12% respectively.

One and two-way analysis of variance (ANOVA) was used to analyze the dose-response relationship and the responses of arterial pressure and heart rate and plasma AVP to norepinephine injection into the PVN.

Baroreflex studies: Male Sprague-Dawley rats weighing 275-350 gram were chronically cannulated as described previously (14,17,35). Briefly, the rats were anesthetized with pentobarbital sodium (45mg/kg, ip), and femoral vein (PE-50) and artery (Dural Plastics) cannulas were placed using sterile procedures. The cannulas were tunneled under the skin of the back and through a spring the was attached to the back of the neck and the top of the cage. All incisions were filled with xylocaine jelly and polysporin (Burroughs-Wellcome) to desensitize the surgical area and to prevent infection. The rats recovered and were caged singly in a room (controlled temperature and humidity) with a 12-h on/off light cycle. All rats had access to food and water ad libitum. After 3 days, the rats were either bilaterally adrenalectomized or sham adrenalectomized under ether anesthesia by the dorsal approach, Corticosterone replacement was effected by placing fused pellets of 20,40, or 80% corticosterone-cholesterol (approx 100mg) or wax pellets under the skin of the back as described by Akana et al. (1). The rats recovered and were all given 0.5% saline to drink and food ad libitum. Six days later, while fully conscious, the arterial cannula was connected to a Statham pressure transducer and polygraph for measurement of arterial blood pressure and heart rate. All cannulas were manipulated outside the cage so as not to disturb the animals. Varying doses of the alpha-1 agonist phenylephrine and nitroglycerin were injected intravenously while monitoring arterial blood pressure and heart rate.

Blood samples were taken before each experiment for determination of plasma corticosterone levels. Plasma corticosterone was determined from heat denatured samples (45) with inter and intraassay coefficients of variation of 8 and 9% respectively. The minimal detectable level in a 10ul sample was 0.1ug/dl.

The responses of heart rate to varying levels of arterial pressure were

compared by using a step-wise polynomial regression while testing the highest coefficient for statistical significance. Coefficients were then compared by t-test.

Responses to Hemorrhage: Male Sprague-Dawley rats were chronically cannulated as described above. Three days later, the rats were either bilaterally adrenalectomized or sham adrenalectomized under ether anesthesia. All rats had access to food and water ad libitum. Five days later, all rats recieved a 15ml/kg*5minute hemorrhage through the arterial cannulae. 2.5ml of blood were taken 20, 60, 120 and 300 minutes after hemorrhage for determination of various plasma constituents. Each blood sample was centrifuged, and the required volume of plasma was removed and put on ice. An equal amount of saline was added to the red cells and whole blood (from hemorrhage) not used for determination of plasma constituents. This saline-red cell mixture was returned to the rat (via the venous cannula) as arterial samples were taken. Arterial blood pressure and heart rate were monitored continuously when samples were not taken.

Plamsa corticosterone, AVP, oxytocin, plasma renin concentration (PRC), norepinephrine, Na^+, K^+, osmolality, protein and gases were determined in this study. Restitution of blood volume was determined by the method of Pirkle and Gann (33). The determination of plasma corticosterone and AVP have been described above. Oxytocin was measured by RIA in acetone extracts from plasma (26). The inter- and intraassay coefficients of variation were 3.2 and 6.8% respectively, and the lowest detectable level was 0.8pg/ml. Plasma renin concentration was determined by adding 0.1ml sample plasma to 0.9ml nephrectomized rat plasma for generation of angiotensin I in vitro at pH 6.5 for 2 hours. Angiotensin I was determined by RIA (38). The inter- and intraassay coefficients of variation were 5.2 and 11.3% respectively and the lowest detectable level was 1ng AI/ml*2h. Plasma norepinephrine was determined by rdioenzymatic assay (32). The inter- and intraassay coefficients of variation were 10.9 and 11.5% respectively. Plasma osmolality, Na^+, K^+ and protein were measured by osmometer, flame photometer, and hand protometer. Arterial PO_2, PCO_2 and pH were measured form a blood gas analyzer.

Two-Way ANOVA was used to compare responses of plasma constituents to hemorrhage. Newman-Keuls multiple range test was used to compared means after ANOVA.

Responses to Hemorrhage, Fed and Fasted: Male Sprague-Dawley rats weighing between 280-350 grams were chronically cannulated and adrenalectomized as described above. Four days after adrenal surgery, food, but not saline, was taken away from half of the rats. On the morning of the 5th day after adrenal surgery, hemorrhage (15 ml/kg*5min) was performed though the arterial cannulae. Further 2.5ml blood samples were withdrawn at 20, 60, 90, 120 and 300 minutes for measurement of Evans' Blue and plasma protein, ACTH, corticosterone, glucose, lactate, B-hydroxybutyrate and alanine. 0.2ml blood samples were additionally taken at 10, 45 and 180 minutes for measurement of plasma glucose, lactate, B-hydroxybutyrate and alanine. The time 0 measurement of these plasma constituents was derived from the hemorrhage blood. The red blood cells derived from the hemorrhage and sampling were mixed with an equivalent volume of saline and reinfused through the venous cannula as blood samples were taken from the arterial cannula.

Arterial blood pressure and heart rate were measured before and after hemorrhage via the arterial cannula as described above. Vascular refilling after hemorrhage was estimated by measuring the dilution of 1mg of Evans' Blue dye intravenously injected before hemorrhage. The concentration of Evans' Blue was

determined at time 0, 20, 60, 90, 120 and 300 minutes after hemorrhage and corrected for loss of Evans' Blue from the vascular space. The loss of Evans' Blue from the vascular space was determined in a separate experiment using eight adrenalectomized and eight Sham-adrenalectomized chronically cannulated rats where 1mg of Evans' Blue dye was injected intravenously and the concentration of dye was determined at the time points stated above. Plasma protein concentration was measured using a hand protometer (National). Plasma glucose concentration was measured by the glucose-oxidase technique (Beckman Glucose Analyzer II). Plasma lactate, B-hydroxybutyrate and alanine concentrations were determined by the Ezymatic Methods of Bergmeyer (5). Plasma ACTH and corticosterone were measured by RIA as described above.

Data were analyzed for statistical significance using a 1-way ANOVA for analysis of response over time and a 2-way ANOVA corrected for repeated measures to compare the responses between groups. Newman-Keuls multiple range test was used to compare means after ANOVA. Significance indicated at $P<0.05$.

Results

Microinjections into the PVN: Because brainstem catecholaminergic cell groups have been implicated in the regulation ACTH and AVP release at the level of the PVN, we have microinjected various catecholamines into the PVN and measured plasma ACTH and AVP. We have found that microinjections of norepinephrine led to a dose- dependent elevation in plasma ACTH (Figure 1) however, unlike L-glutamate which not only elevated ACTH and AVP but also caused a potent bradycardia (16), microinjections of norepinephrine had no effect on heart rate or arterial blood pressure. Also, microinjection of 10^{-4}M norepinephrine into the PVN caused a rise in plasma AVP. Control injections of vehicle and injections of norepinephrine outside the nucleus had no effect (Figure 2).

Baroreflex studies: Brainstem catecholaminergic cell groups have also been implicated in the regulation of baroreflex function and these neurons have been shown to contain type II glucocorticoid receptors. To determine if loss and replacement of glucocorticoids affects baroreflex function, we recorded the changes in heart rate while increasing and decreasing MABP with varying doses of phenylephrine and nitroglycerine in conscious rats that were adrenalectomized and given corticosterone (in pellet form, 0, 20, 40 and 80% mixed with cholesterol) at the time of adrenal surgery. Baroreflex curves were determined and it was found that the curves were significantly different between ADRX and Sham rats (Figure 3). We also found that the best replacement dose that corrected the reflex curve was the ADRX+80% pellet group (corticosterone levels of 5.1 ± 0.4 ug/dl) while the 40% and 20% replacement groups (2.1 ± 0.5 and 1.0 ± 0.2 ug/dl, respectively) had baroreflex curves that were not different from ADRX+0%. The levels of corticosterone (80% group) that were necessary to restore baro-function were significantly higher then those needed to restore body weight, thymus weight and plasma CBG levels to normal (40% pellet). Upon further analysis of the baroreflex curves, it was found that the slopes of the regressions though the pnenylephrine side of the curves (increase in MABP) were not different between groups (Table 1, Figure 4, solid lines); however, the slopes of the nitroglycerine part of the curves (dashed lines) were different between the Sham and ADRX+0% groups; the ADRX+80% pellet group had a slope not different from sham. This suggests that the baroreflex may be regulated by two systems. One that regulates heart rate during hypertension and one that regulates heart rate during hypotension. It is the latter system that appears to be affected by glucocorticoids.

Responses to Hemorrhage: Since brainstem catecholaminergic groups contain glucocorticoid receptors and are located in areas involved in the regulation of arterial blood pressure during hypovolemia, we have studied the recovery of arterial blood pressure and have characterized the responses of various hormones and blood constituents after hemorrhage in adrenalectomized rats. These have been difficult studies to perform in the past because adrenally insufficient animals have a very high mortality rate. However, we have developed a conscious adrenalectomized model that is healthy and viable.

We have now shown that the recovery of mean arterial blood pressure (MABP) to 15ml/kg*5min hemorrhage in the adrenalectomized (ADRX) rat is almost identical to that in sham rats (Figure 5). This was surprising since there is a large literature demonstrating that ADRX rats, cats, dogs, and adrenally insufficient humans are very fragile and do not survive this type of stress. Further studies in this lab have revealed that the recovery of MABP in ADRX rats is probably due to the potentiated recovery of heart rate (Figure 5), and the potentiated responses of vasoactive hormones: vasopressin, oxytocin, renin and norepinephrine (Figure 6). These data suggest that some component, possibly a neural one, has changed the gain of the system. Restitution of plasma volume in the ADRX rats lagged behind that of the Sham rats, which was due, in part, to the difference in the response of plasma osmolality (Figure 7). Restitution of plasma proteins was not different between groups (Figure 7), nor was there a difference in the responses of pH, PCO_2, PO_2 (Figure 8).

Responses to Hemorrhage, Fed and Fasted: Areas in the brainstem that contain catecholaminergic neurons have also been implicated in the regulation of feeding and nutrition. Glucocorticoids have long been implicated in the regulation of metabolism though various central structures. These studies were performed to characterize the responses of the cardiovascular and hormonal systems to hemorrhage in fed and fasted conscious rats that were given food ad libitum or were fasted for the 20-24h prior to hemorrhage.

In agreement with our previous finding (above), all fed, adrenalectomized rats lived though the hemorrhage and subsequent 24 hours. By contrast, all fasted adrenalectomized rats died within 24 hours of the hemorrhage, most of them between 2.5 and 3.5 hours after the hemorrhage volume was removed. The responses in MABP and heart rate in both fed and fasted adrenalectomized and sham-adrenalectomized rats are shown in Figure 9. Fed, adrenalectomized rats restored and maintained MABP well compared to shams. By contrast, the adrenalectomized rats did not sustain MABP, demonstrating a slow decline until death occurred even though the initial return of MABP resembled the return of MABP in the fasted sham controls during the first hour. Initial heart rate was elevated in both groups of adrenalectomized rats compared to the sham controls. After the reflex bradycardia occasioned by the hemorrhage, heart rate continued to be elevated compared to shams for several hours.

The dilution of Evans' Blue dye (which reflects both return of fluid into the vascular system via Starling forces across the capillaries and successive dilution of plasma protein by the repeated removal of plasma and replacement with saline) did not differ between sham adrenalectomized and adrenalectomized rats under either fed or fasted conditions (Figure 10, left and right). The initial concentration of Evans'Blue was greater in the fasted than the fed rats, reflecting the decrease in blood volume that occurs with a 20-24 hour fast.

The responses of plasma ACTH and corticosterone to hemorrhage are shown in Figure 11. All groups demonstrated an elevation of plasma ACTH to hemorrhage from basal levels. Initial ACTH concentrations were elevated in the

adrenalectomized rats compared to the shams (Fig. 11, top), and there was no corticosterone response to the hemorrhage in these groups (Fig. 11, bottom). By contrast, the magnitudes of the ACTH responses to hemorrhage were similar in fed and fasted, adrenalectomized and sham adrenalectomized rats suggesting that the neural elements that regulate ACTH release have changed their operating set point and not the gain of the system. The ACTH response persisted for approximately 2 hours and hormone concentrations had returned to initial levels by 5 hours in the rats that survived.

Plasma concentrations of energy substrates are shown in Figure 12. In fed, sham adrenalectomized controls, plasma glucose concentrations did not change after hemorrhage (Fig 12, top panel). By contrast, in fed, adrenalectomized rats glucose concentrations fell between 1 and 2 hours and remained at a plateau thereafter. Initial plasma glucose concentrations were decreased in both groups of fasted rats. Sham adrenalectomized rats increased their plasma glucose levels with time after hemorrhage so that by 5 hours the levels were elevated above initial values. Plasma glucose levels fell in fasted adrenalectomized rats, and from 90 minutes until death there was a progressive and marked decline in these levels.

Plasma lactate concentration demonstrated a biphasic increase to hemorrhage in all groups (Figure 12, 2nd panel). However, the fasted adrenalectomized group showed an exaggerated response, compared to the fasted sham group. Plasma B-hydroxybutyrate concentrations did not change with time after hemorrhage in the fed groups. By contrast, fasting resulted in elevated initial values of this fatty acid, and hemorrhage occasioned an increase in the sham adrenalectomized, but not the adrenalectomized rats (Figure 12, third panel, left and right). Plasma concentrations of alanine were only marginally affected in these experiments, with minor elevations occurring in the fasted adrenalectomized rats at 90 and 180 minutes (Figure 12, bottom panel, left and right).

Discussion

Microinjections into the PVN: There is both anatomic and physiologic evidence for the role of central catecholaminergic regulation of basal and stress induced release ACTH and AVP. Catecholaminergic pathways between the nucleus of the NTS and PVN have previously been described and include 1) noradrenergic and adrenergic cell bodies in the caudal extent of the NTS (A2,C2 cell groups) and 2) the A1,C1, A5 and A6 cell groups (9,10,37,39). All these groups receive hemodynamic information (changes in blood pressure and blood volume) and project to the PVN. Thus, the anatomical evidence for catecholamines regulating ACTH and AVP release is strong.

Physiologic evidence for catecholaminergic regulation of the adrenocortical system is also strong. Destruction of catecholamine cell groups by central injection of 6-hydroxydopamine (6-OHDA) results in disruption of both basal and stress-induced activity in the adrenocortical system (see Review in 12). Moreover, catecholamines injected into the brain elevate plasma ACTH (34,40) and AVP (4). However, it is not clear what receptor type is involved or if the PVN is the site of action of the catecholamines.

Figures 1 demonstrates that norepinephrine microinjected directly into the PVN not only leads to an elevation in plasma ACTH, but does so in a dose-related manner. Also, norepinephrine microinjected directly into the PVN leads to a rise in plasma AVP (Figure 2)

Baroreflex studies: Glucocorticoid receptors have been found in cell located in

medullary structures known to mediate baroreceptor signals and regulate sympathetic output (22). These data demonstrate that complete loss of corticosterone alters barofunction (Figures 3,4 and Table 1) and that only the high 80% corticosterone pellet normalized the deficit. Since the normal circadian rhythm for corticosterone in rats ranges from 0.5$\pm$0.1ug/dl in the morning to 9.0$\pm$2.3ug/dl in the evening (as measured in these rats), the results in Figures 3 and 4 suggest that it is the evening rise in corticosterone that allows normal baroreceptor function. Also, it appears that adrenalectomized rats have an abnormal nitroglycerin-induced baroreceptor curve while the

phenylephrine-induced curve was normal (Figure 4, Table 1). This suggests that corticosterone affects only one limb of the baroreceptor curve, i.e., the regulation of heart rate as pressure falls.

Responses to Hemorrhage: These results demonstrate that recovery of MABP in the adrenalectomized (ADRX) conscious rat is similar to that in the Sham rat (Figure 5); however, responses in heart rate, vasopressin, oxytocin, renin and norepinephrine were all potentiated. The restitution of blood volume in the ADRX rat, although lagging behind that of the sham group, was over 100% by 5 hours. This lag in the restitution of blood volume in the ADRX group may be due to the fall in plasma osmolality in the ADRX group instead of a rise (demonstrated by the Sham group) and not due to a deficit in plasma protein restitution since its recovery was not different from that measured in the Sham group.

Under normal conditions, the recovery of MABP after hemorrhage involves brain stem centers that coordinate actions of the autonomic nervous system and the pituitary for regulation of vasoconstriction and fluid homeostasis. These results show that conscious ADRX rats can regulate arterial blood pressure at a level similar to that in Sham rats, even though there is a lag in the restitution of blood volume, by elevating heart rate (and thereby cardiac output) and vasoactive hormones (Figure 6).

Responses to Hemorrhage, Fed and Fasted: Fed and fasted sham-adrenalectomized (control) rats restored arterial blood pressure to near normal values within 60 minutes of hemorrhage, and sustained this over the 5 hour period of study (Figure 1). Although plasma glucose levels were fairly constant in the fed control rats, glucose production appeared to have been increased in the fasted rats after 2 hours. Similarly, although plasma B-hydroxybutyrate concentrations in the fed control rats remained fairly stable, there tended to be an increase in the concentrations of this energy substrate during the first 2 hours after hemorrhage (Figure 4). Thus, the fasted control animals appeared to mobilize substrate after hemorrhage, first in the form of fatty acids and subsequently in the form of glucose. The timing of such responses is compatible with the known actions of elevated levels of vasopressin, norepinephrine and glucocorticoids secreted in response to the hemorrhage (15,18,29). The fact that responses of similar magnitude in circulating energy substrate levels did not occur in the fed controls suggests that the hormonal responses were counteracted by other means.

The major differences in responses between fed and fasted adrenalectomized rats which we measured, were those that began to occur 60 minute after hemorrhage. At about this time, both MABP and plasma glucose levels began to decline slowly in the fasted, adrenalectomized rats. The decline in these variables was sustained for the next few hours, until death usually occurred. From our data, the cause of death could have been equally well hypoglycemia or circulatory failure. Plasma B-hydroxybutyrate levels did not rise in the fasted adrenalectomized rats as they did in the fasted controls, but instead declined slowly during the post-hemorrhage period. The lowest level of this substrate observed in adrenalectomized rats was equivalent to concentrations in the fed animals. Since muscle preferentially uses fatty acids for energy under conditions of starvation, it may be that the slow fall in this substrate in fasted adrenalectomized rats was also a factor contributing to death.

The finding that there is a strong interaction between feeding state and adrenalectomy on survival after hemorrhage stress, strengthens our previously articulated hypothesis that a primary phyiological role of the adrenocortical system is to serve as an efferent arm of a larger hypothalamic system involved

with calorie intake, storage and mobilization (11). The activity of the adrenocortical system is quantitatively responsive to caloric restriction (23,24,43,44), and death that occurs after hemorrhage in fasted, adrenalectomized rats, but not fasted controls, suggests that the fasting-induced rise in corticosteroids may play a life-saving role in the capacity to withstand stress.

Bibliography

1) Akana SF, Cascio CS, Shinsako J, Dallman MF 1985 Corticosterone:narrow range required for normal body and thymus weight and ACTH. Am. J. Phyiol. 249:R527.

2) Al-Damluji S 1988 Adrenergic mechanisms in the control of corticotrophin secretion. J. Endocr. 119:5.

3) Antoni FA 1986 Hypothalamic control of adrenocorticotropin secretion: Advances since the discovery of 41-residue corticotropin-releasing factor. 7:351.

4) Benetos A, Gavras I, Gavras H 1986 Norepinephrine applied in the paraventricular hypothalamic nucleus stimulates vasopressin release. 381:322.

5) Bergmeyer HU, Editor-in-Chief, Methods of Enzymatic Analysis. 1974, 2nd ed., VCH Publishers, Deerfield Beach FL, pp1464-1483, pp1679-1682, pp1836-1840.

6) Beyer HS, Matta SG, Sharp BM 1988 Regulation of messenger ribonucleic acid for corticotropin-releasing factor in the paraventricular nucleus and other brain sites of the rat. Endocrinology 123:2117.

7) Bloom FE, Battenberg ELF, Rivier J, Vale W 1982 corticotropin-releasing factor (CRF) immunoreactive neurons and fibers in rat hypothalamus. Reg. Peptides 4:43.

8) Bugnon CD, Fellman D, Gouget A 1983 Changes in corticotropin and vasopressin-like immunoreactivities in the zona externa of the median eminence in adenalectomized rats: Immunocytochemical study. Neurosci. Lett. 37:43.

9) Ciriello J, Calaresu FR 1980 Autoradiographic study of ascending projections from cardiovascular sites in the nucleus tractus solitarii in the cat. Brain Res. 196:448.

10) Ciriello J, Calaresu FR 1980 Monosynaptic pathway from cardiovascular neurons in the nucleus tractus solitarii to the paraventricular nucleus of the cat. Brain Res. 193:529.

11) Dallman MF 1984 Viewing the ventromedial hypothalamus from the adrenal gland. Am. J. Physiol. 246:R1.

12) Dallman MF, Akana SF, Cascio CS, Darlington DN, Jacobson L, Levin N 1987 Regulation of ACTH secretion: Variations on a theme of B. Rec. Prog. Horm. Res. 43:113.

13) Dallman MF, DeManicor D, Shinsako J 1974 Diminishing corticotrope capacity to release ACTH during sustained stimulation: the twenty four hours after bilateral adrenalectomy in the rat. Endocrinology 95:65.

14) Darlington DN, Kaship K, Keil LC, Dallman MF 1989 Vascular responsiveness in adrenalectomized rats with corticosterone replacement. Am. J. Physiol. 256:1274.

15) Darlington DN, Keil LC, Dallman MF 1989 Potentiation of hormonal responses

and fasting, but not hypoglycemia in conscious adrenalectomized rats. Endocrinology 125:1398.

16) Darlington DN, Miyamoto M, Keil LC, Dallman MF 1989 Paraventricular stimulation with glutamate elicits bradycardia and pituitary responses. Am. J. Physiol. 256:R112.

17) Darlington DN, Shinsako J and Dallman MF 1986 Medullary lesions eliminate ACTH responses to hypotensive hemorrhage. Am. J. Physiol. 251:R106.

18) Darlington DN, Shinsako J and Dallman MF 1988 Paraventricular lesions: Hormonal and cardiovascular responses to hemorrhage. Brain Res. 439:289.

19) Davis LG, Arentzen R, Reid JM, Manning RW, Wolfson B, Lawrence KL, Baldino F 1986 Glucocorticoid sensitivity of vasopressin mRNA levles in the paraventricular nucleus of the rat. Proc. Natl. Acad. Sci. 83:1145.

20) Dornhorst A, Carlson DE, Seif SM, Robinson AG, Zimmerman EA, Gann DS 1981 Control of release of adrenocorticotropin and vasopressin by the supraoptic and paraventricular nuclei. Endocrinology 108:1420.

21) Gann DS, Pirkle JC 1975 Role of cortisol in the restitution of blood volume after hemorrhage. Am. J. Surg. 130:565.

22) Harfstrand A, Fuxe K, Cintra A, Agnati LF, Zini I, Wikstrom A, Okret S, Yu Z, Goldstein M, Steinbusch H, Verhofstad A, Gustafsson J 1986 Glucocorticoid receptor immunoreactivity in monoaminergic neurons of rat brain. Proc. Natl. Acad. Sci. 83:9779.

23) Honma S, Honma K, Nagasaka T, Hiroshige T 1987 The ventromedial hypothalamic nucleus is not essential for the prefeeding corticosterone peak in rats under restricted daily feeding. Physiology and Behavior 39:211

24) Honma K, Honma S, Hirai T, Datsuno Y, Hiroshige T 1986 Food ingestion is more important to plasma corticosterone dynamics than water intake in rats under restricted daily feeding. Physiology and Behavior 37:791.

25) Hyde TM, Miselis RR 1983 Effects of area postrema/caudal medial nucleus of solitary tract lesions on food intake and body weight. Am. J. Physiol. 244:R577.

26) Keil LC, Rosella-Dampman M, Emmert S, Chee O, Summy-Long JY 1984 Enkephalin inhibition of angiotensin-stimulated release of oxytocin and vasopressin. Brain Res. 297:329.

27) Kovacs K, Kiss JZ, Makara GB 1986 Glucocorticoid implants around the hypothalamic paraventricular nucleus prevent the increase of corticotropin-releasing factor and arginine vasopressin immunostaining induced by adrenalectomy. Neuroendocrinology 44:229.

28) Leibowitz SF 1980 Neurochemical systems of the hypothalamus: Control of feeding and drinking behavior and water-electrolyte excretion. In: Handbook of The Hypothalamus. eds. Morgan PT and Pauskepp J, Marcel Dekker Inc NY pp299.

29) Lilly MP, Engeland WC, Gann DS 1986 Adrenal medullary responses to repeated hemorrhage in conscious dogs. Am. J. Physiol. 251:R1193.

30) Liposits ZS, Phelix C, Paull WK 1986 Adrenergic innervation of corticotropin releaseing factor (CRF)-synthesizing neurons in the hypothalamic paraventricular nucleus of the rat. Histochemistry 84:201.

31) Norgren R 1978 Projections of the nucleus of the solitary tract in the rat. Neuroscience 3:207.

32) Peuler JD, Johnson GA 1977 Simultaneous single isotope radioenzymatic assay of plasma norepinephrine, epinephrine and dopamine. Life Sci 21:625.

33) Pirkle JC, Gann DS, Allen-Rolands CF 1982 role of the pituitary in restitution of blood volume after hemorrhage. Endocrinology 110:7.

34) Plotsky PM 1987 Facilitation of immunoreactive corticotropin-releasing factor secretion into the hypophysial-portal circulation after activation of catecholaminergic pathways or central norepinephrine injection. Endocrinology 121:924.

35) Raff H, Fagin KD 1984 Measurement of hormones and blood gases during hypoxia in conscious cannulated rats. Am. J. Physiol. 252:R635.

36) Robertson GL 1978 Posterior Pituitary, In: Endocrinology and Metabolism. ed. P. Felig, J.D. Baxter, A.E Braodus, L.A. Frohman, 2nd ed., pp338.

37) Sawchenko PE, Swanson LW 1981 Central noradrenergic pathways for the integration of hypothalamic neuroendocrine and autonomic responses. Science 214:685.

38) Stockigt JR, Collins RD, Biglieri EG 1971 Determination of plasma renin concentration by angiotensin I immunoassay. Diagnostic import of precise measurement of subnornal renin in hyperaldosteronism. Circ. Res. 28/29, Suppl. II:175.

39) Swanson LW, Kuypers HGJM 1980 The paraventricular nucleus of the hypothalamus: cytoarchitectonic subdivisions and the organization of projections to the pituitary, dorsal vagal complex and spinal cord as demonstrated by retrograde fluorescence double-labeling methods. J. Comp. Neurol. 194:555.

40) Szafarczyk A, Malaval F, Laurent A, Gibaud R, Assenmacher I 1987 Further evidence for a central stimulatory action of catecholamines on adrenocorticotropin release in the rat. Endocrinology 121:883.

41) Thrivikraman KV, Bereiter DA, Gann DS 1989 Catecholamine activity in paraventricular hypothalamus after hemorrhage in cats. Am. J. Physiol. 257:R370.

42) Wade CE, Bie P, Keil LC, Ramsay DJ 1982 Osmotic control of plasma vasopressin in the dog. Am. J. Physiol. 243:E287.

43) Webb WR, Degerli IU, Hardy JD, Unal M 1965 Cardiovascular responses in adrenal insufficiency. Surgery 58:273.

44) Wilkinson CW, Shinsako J, Dallman MF 1979 Daily rhythms in adrenal responsiveness to adrenocorticotropin are determined primarily by the time of feeding in the rat. Endocrinology 104:350.

45) Wilkinson CW, Shinsako J, Dallman MF 1981 Return of pituitary-adrenal function after enucleation or transplantation: diurnal rhythms and responses to ether. Endocrinology 109:162.

Table 1 Slopes from linear regression analysis

	Nitroglycerin	Phenylephrine
Sham	-2.22+0.17	-1.22+0.07
ADRX+0%	-1.27+0.11*	-1.10+0.19
ADRX+20%	-1.62+0.22*	-1.41+0.07
ADRX+40%	-1.27+0.20*	-1.12+0.07
ADRX+80%	-2.00+0.14	-1.24+0.06

Slope calculated from nitroglycerin-induced and phenylephrine-induced changes in mean arterial blood pressure vs. changes in heart rate. ADRX, adrenalectomized. *$P < 0.05$ compared to Sham group by t-test.

Figure Legends

Figure 1. Dose-response curve for plasma ACTH after microinjection (50nl) of varying concentrations of norepinephrine into the paraventricular nucleus of the hypothalamus in pentobarbital-anesthetized rats.

Figure 2. Response of vasopressin (AVP) after injection of norepinephrine into the PVN, or into structures surrounding the PVN (Miss) or injections of vehicle (Sham).

Figure 3. Baroreflex curves in conscious adrenalectomized (ADRX) rats without and with corticosterone replacement (pellets 0,20,40 and 80% mixed with cholesterol). Heart rate was recorded as mean arterial blood pressure was manipulated by bolus injections of varying doses of phenylephrine or nitroglycerin. Simple linear regressions best fit the ADRX+0, 20 and 40% data. By contrast, quadratic functions best fit the data from Sham and ADRX+80% groups; the coefficients were not different between Sham and ADRX+80% groups, strongly suggesting the responses were the same.

Figure 4. Further analysis of baroreflex curves showing linear regressions for data generated from phenylephrine-induced increases in arterial blood pressure (solid line) and nitroglycerin-induced decreases in arterial blood pressure. Slopes from the regression analysis are in Table 1.

Figure 5. The responses of mean arterial blood pressure (MABP) and heart rate to 15ml/kg*5min hemorrhage in conscious adrenalectomized (ADRX) and sham-adrenalectomized rats.

Figure 6. The responses of plasma corticosterone (ug/dl), vasopressin (AVP, pg/ml), oxytocin (OXY, pg/ml), plasma renin concentration (PRC, mg AII/ml), and norepinephrine (norepi, pg/ml) after 15ml/kg*5min in conscious ADRX and Sham rats.

Figure 7. The responses of plasma Na+ (mEquil/l), K+ (mEquil/l) and osmolality (mOsmol/kg) and % restitution of plasma protein and blood volume after 15ml/kg*5min hemorrhage in conscious ADRX and Sham rats.

Figure 8. The responses of PO_2, PCO_2 and pH to 15ml/kg*5min hemorrhage in conscious ADRX and Sham rats.

Figure 9. The responses of mean arterial blood pressure (MABP) and Heart Rate (HR) to 15ml/kg*5minute hemorrhage in conscious fed and 20-24hr fasted sham-adrenalectomized (Sham) and adrenalectomized (ADRX) rats. There was a significant difference (2-Way ANOVA) between the Sham and ADRX groups for the responses of MABP and HR (for both fed and fasted treatments). Values represent means$\pm$SE.

Figure 10. The dilution of Evans' Blue dye (1mg) in plasma after 15ml/kg*5minute hemorrhage in conscious fed and 20-24hr fasted sham-adrenalectomized (Sham) and adrenalectomized (ADRX). There was no significant difference between groups. Values represent means$\pm$SE.

Figure 11. The responses of plasma ACTH and corticosterone after 15ml/kg*5minute hemorrhage in conscious fed and 20-24hr fasted sham-adrenalectomized (Sham) and adrenalectomized (ADRX). There was a significant difference (2-Way ANOVA) between the Sham and ADRX groups for the responses of ACTH and corticosterone (for both fed and fasted treatments). Values represent means$\pm$SE.

Figure 12. The responses of plasma glucose, lactate, B-hydroxybutyrate and l-alanine after 15ml/kg*5minute hemorrhage in conscious fed and 20-24hr fasted sham-adrenalectomized (Sham) and adrenalectomized (ADRX). There was a significant difference (2-Way ANOVA) between the Fasted Sham and ADRX groups for the responses of all plasma substrates and between the fed Sham and ADRX groups for the responses of glucose. * Significant difference at that time point by Newman-Keuls. Values represent means$\pm$SE.

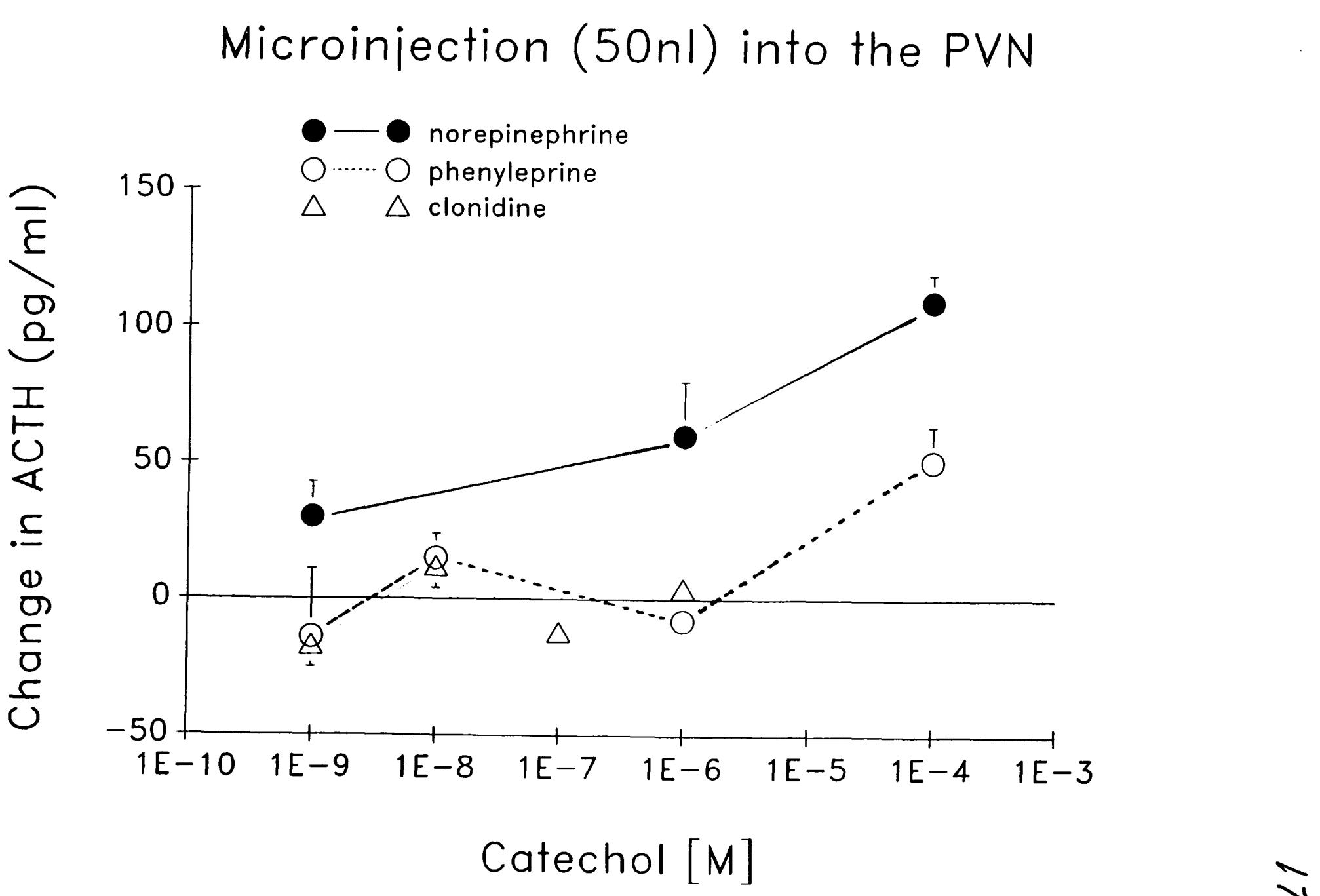
Microinjection (50nl) into the PVN
norepinephrine
phenyleprine
clonidine
Change in ACTH (pg/ml)
150
100
50
0
−50
1E−10
1E−9
1E−8
1E−7
1E−6
1E−5
1E−4
1E−3
Catechol [M]

Figure 2

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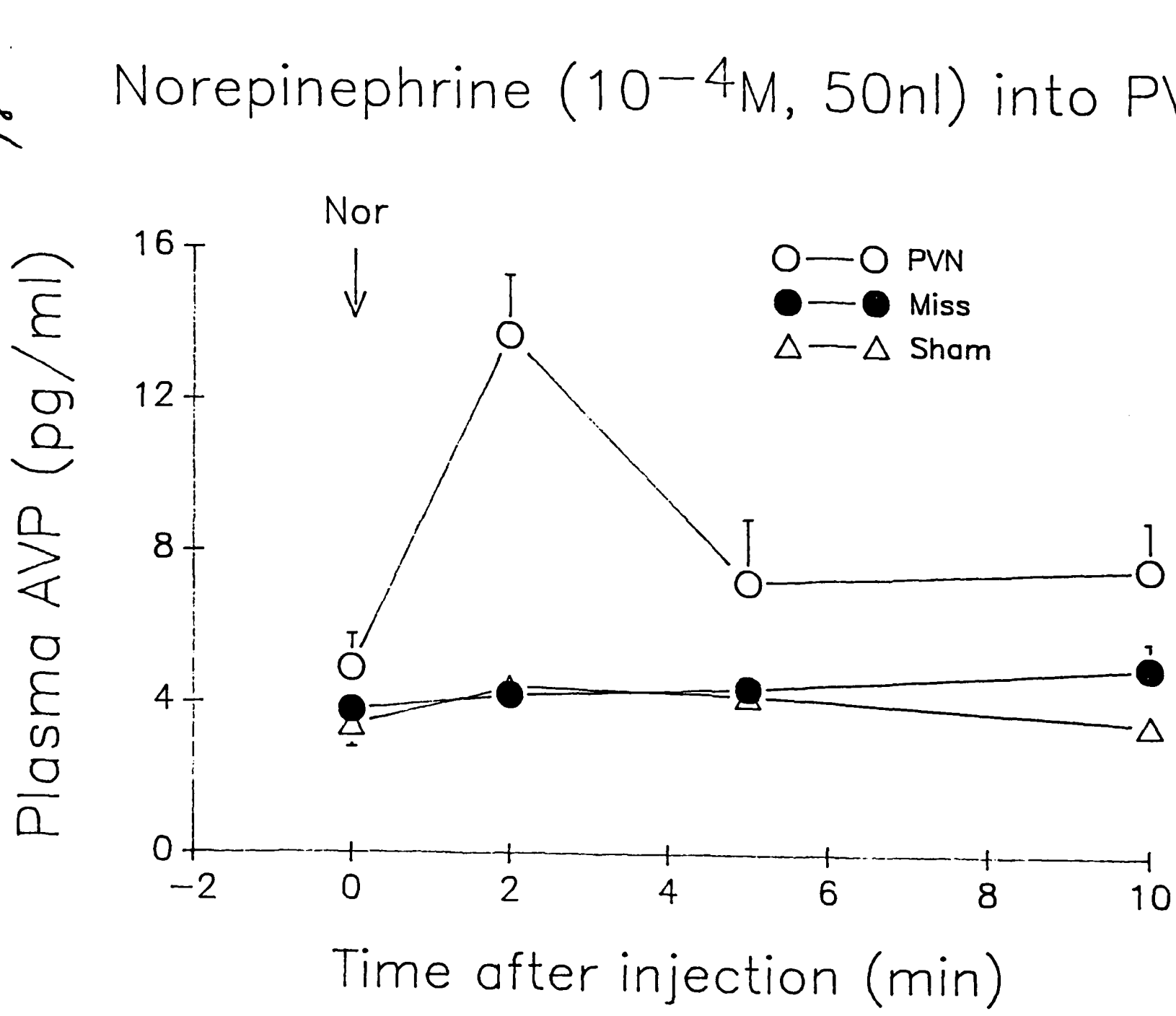

Norepinephrine (10^{-4}M, 50nl) into PVN
Nor
PVN
Miss
Sham
Plasma AVP (pg/ml)
16
12
8
4
0
−2
0
2
4
6
8
10
Time after injection (min)

Figure 3

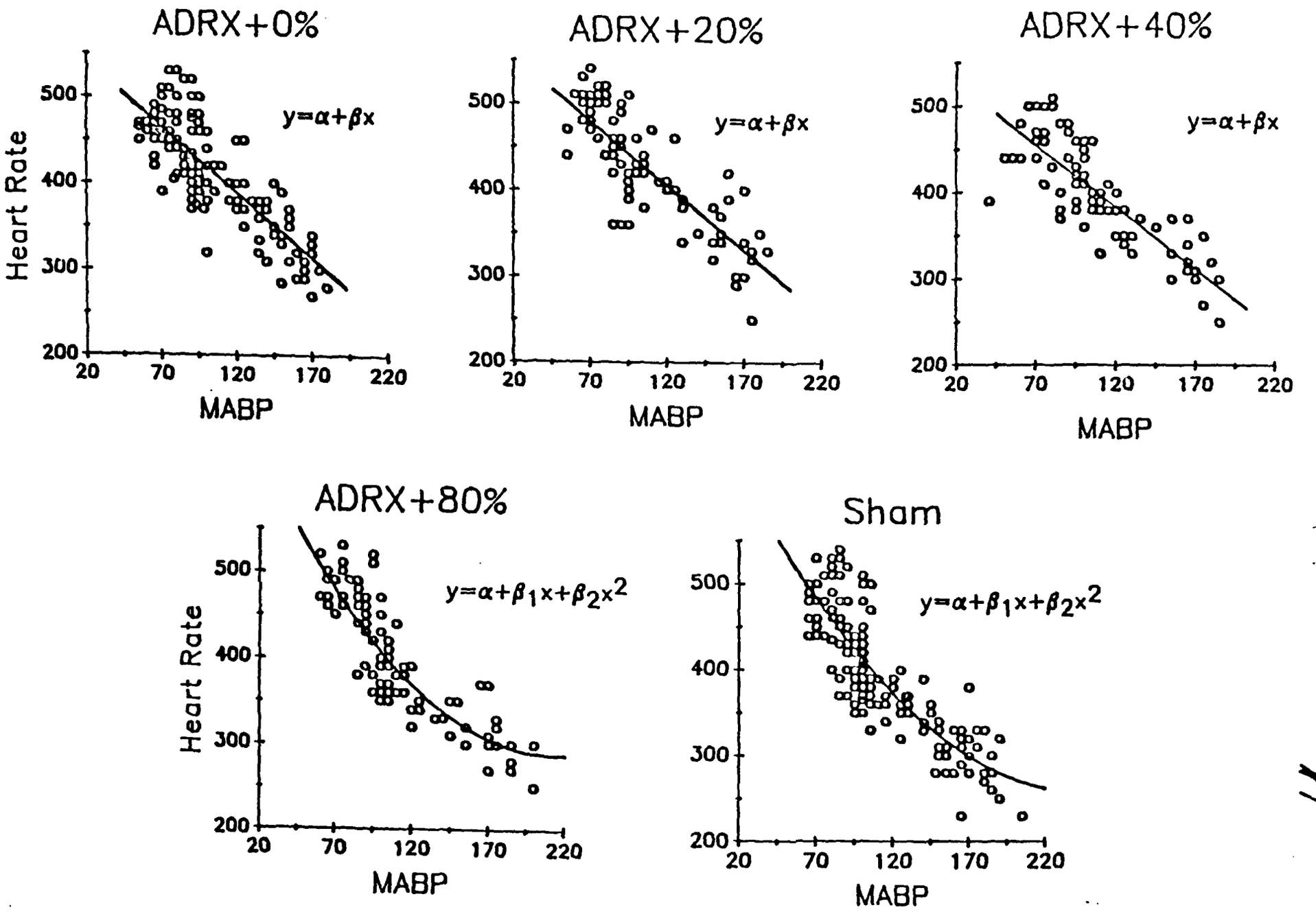

ADRX+0%
ADRX+20%
ADRX+40%
ADRX+80%
Sham
Heart Rate
MABP
y=α+βx
y=α+β1x+β2x2
500
400
300
200
20
70
120
170
220

Figure 4

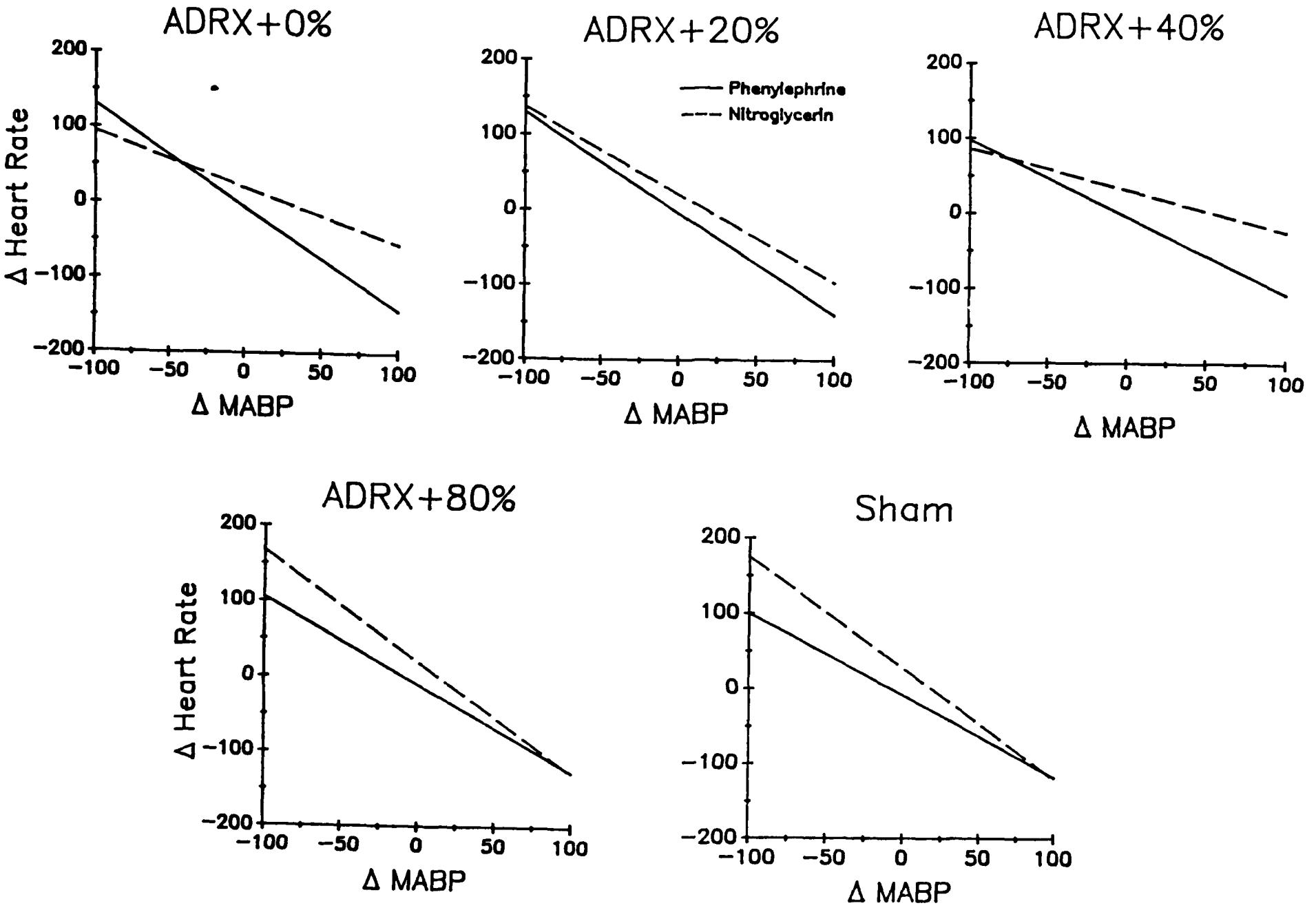

ADRX+0%
ADRX+20%
ADRX+40%
ADRX+80%
Sham
Phenylephrine
Nitroglycerin
Δ Heart Rate
Δ MABP
200
100
0
−100
−200
−100
−50
0
50
100

Figure 5

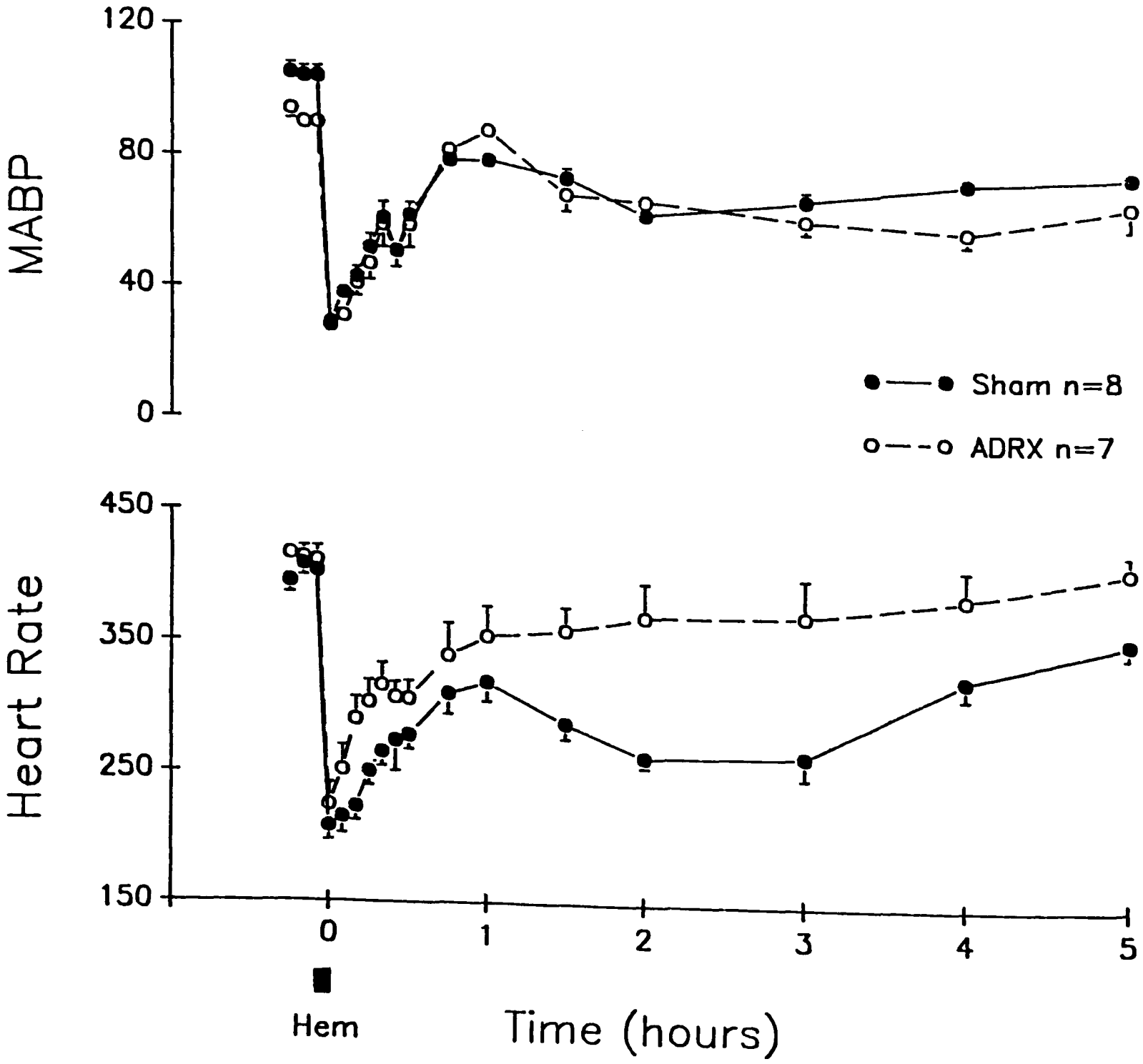

MABP
120
80
40
0
Sham n=8
ADRX n=7
Heart Rate
450
350
250
150
0
1
2
3
4
5
Hem
Time (hours)

Figure 6

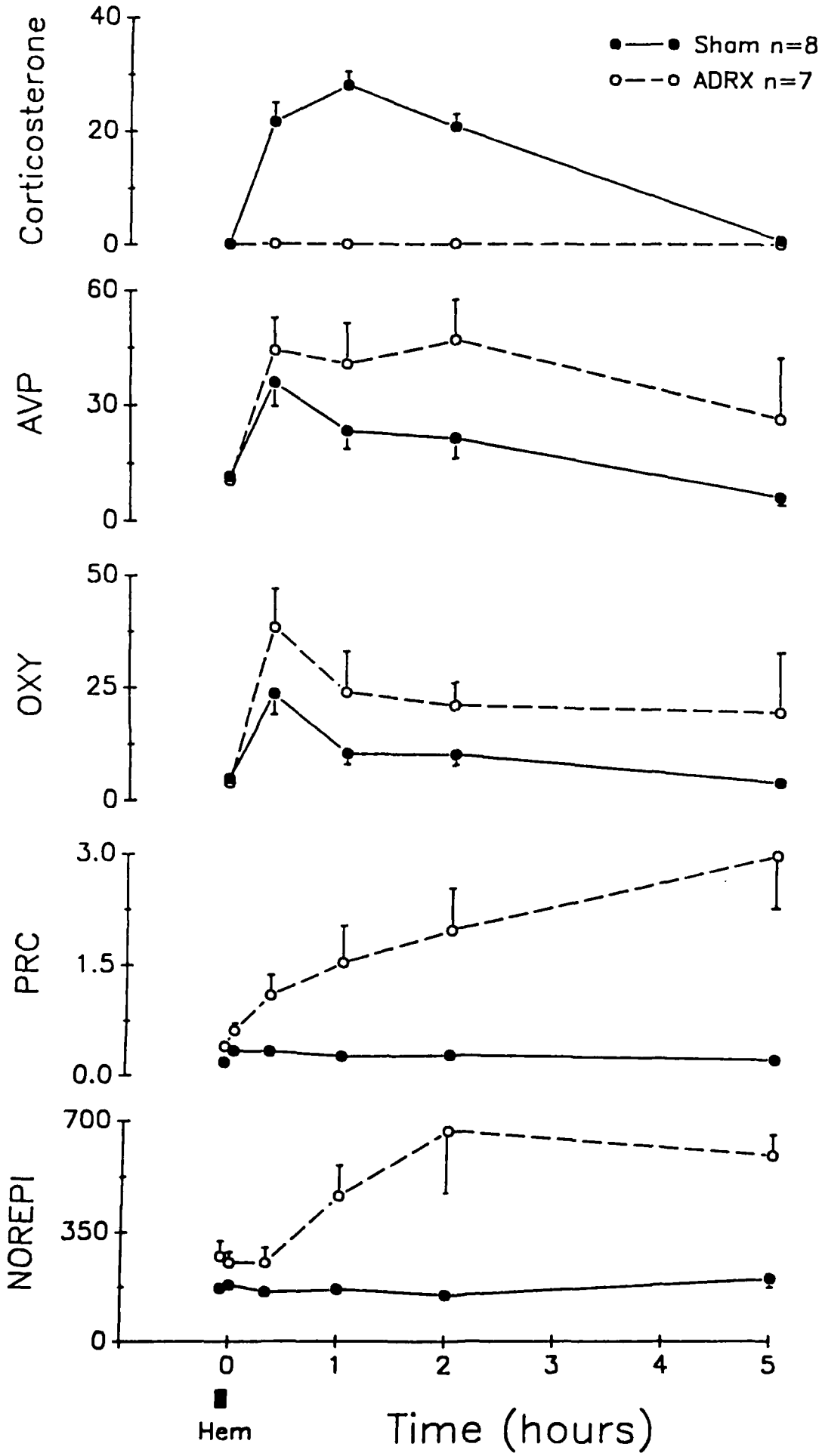

Corticosterone
40
20
0
Sham n=8
ADRX n=7
AVP
60
30
0
OXY
50
25
0
PRC
3.0
1.5
0.0
NOREPI
700
350
0
0
1
2
3
4
5
Hem
Time (hours)

Figure 7

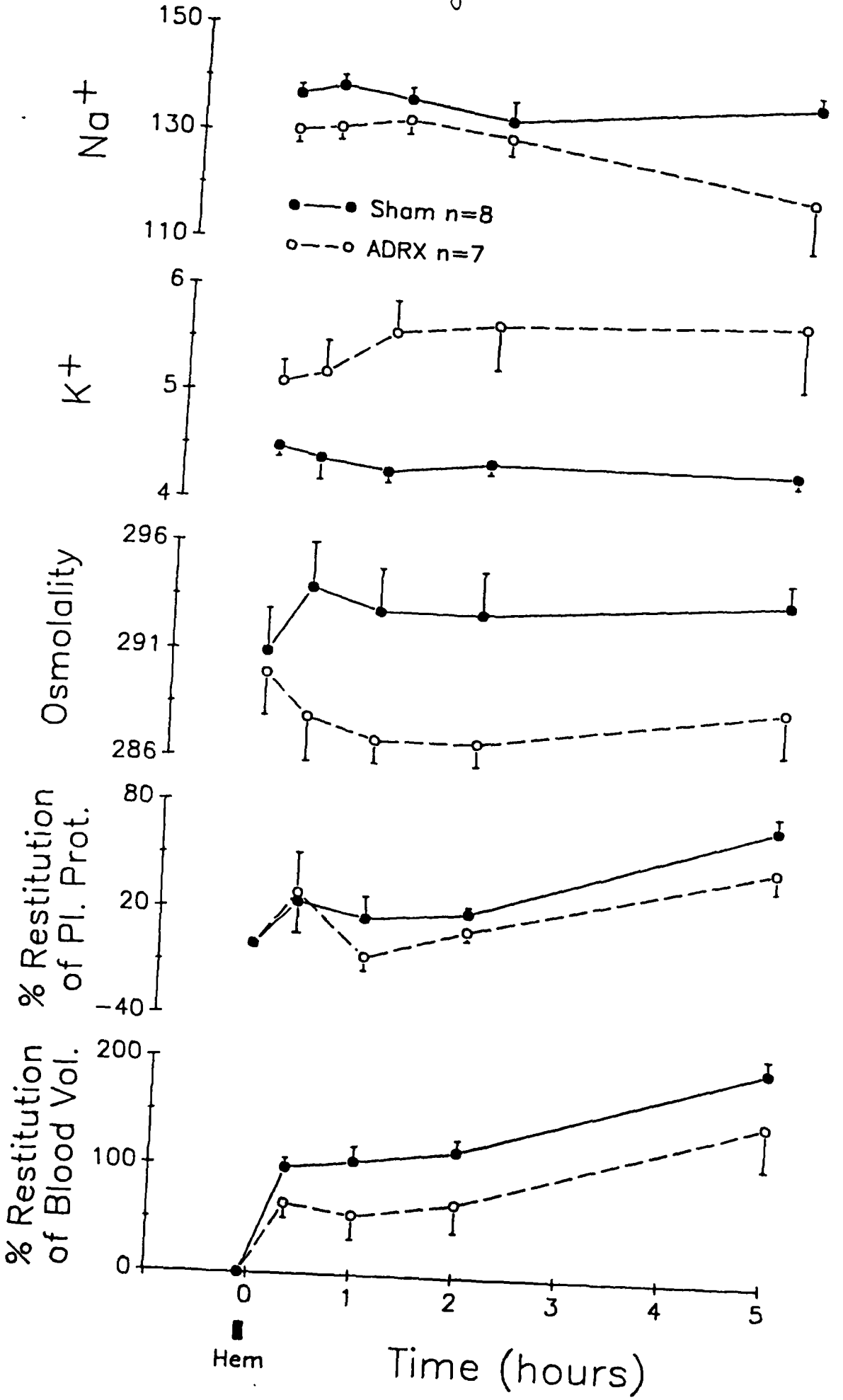

150
130
110
Na+
Sham n=8
ADRX n=7
6
5
4
K+
296
291
286
Osmolality
80
20
-40
% Restitution of Pl. Prot.
200
100
0
% Restitution of Blood Vol.
0
1
2
3
4
5
Hem
Time (hours)

Figure 8

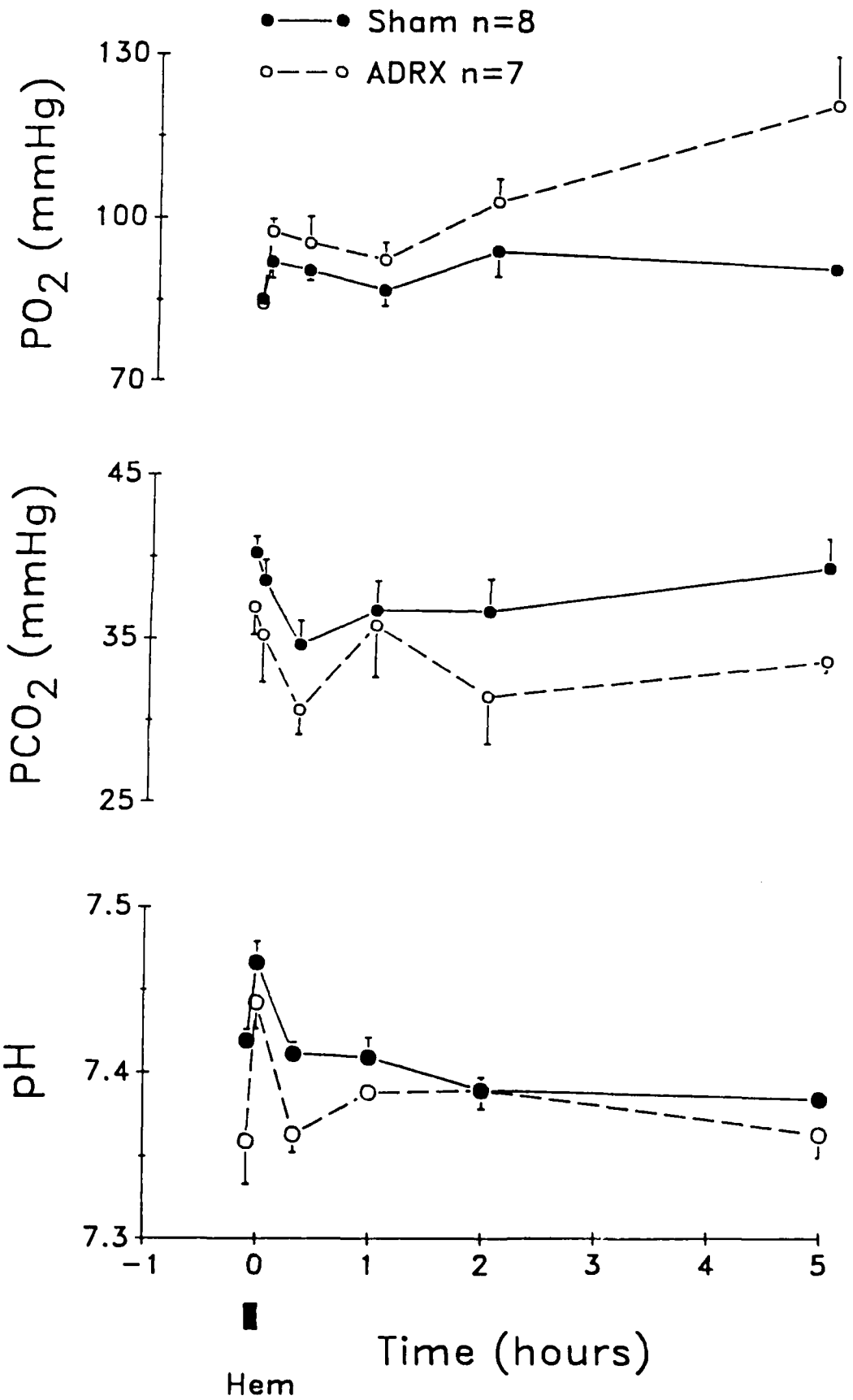
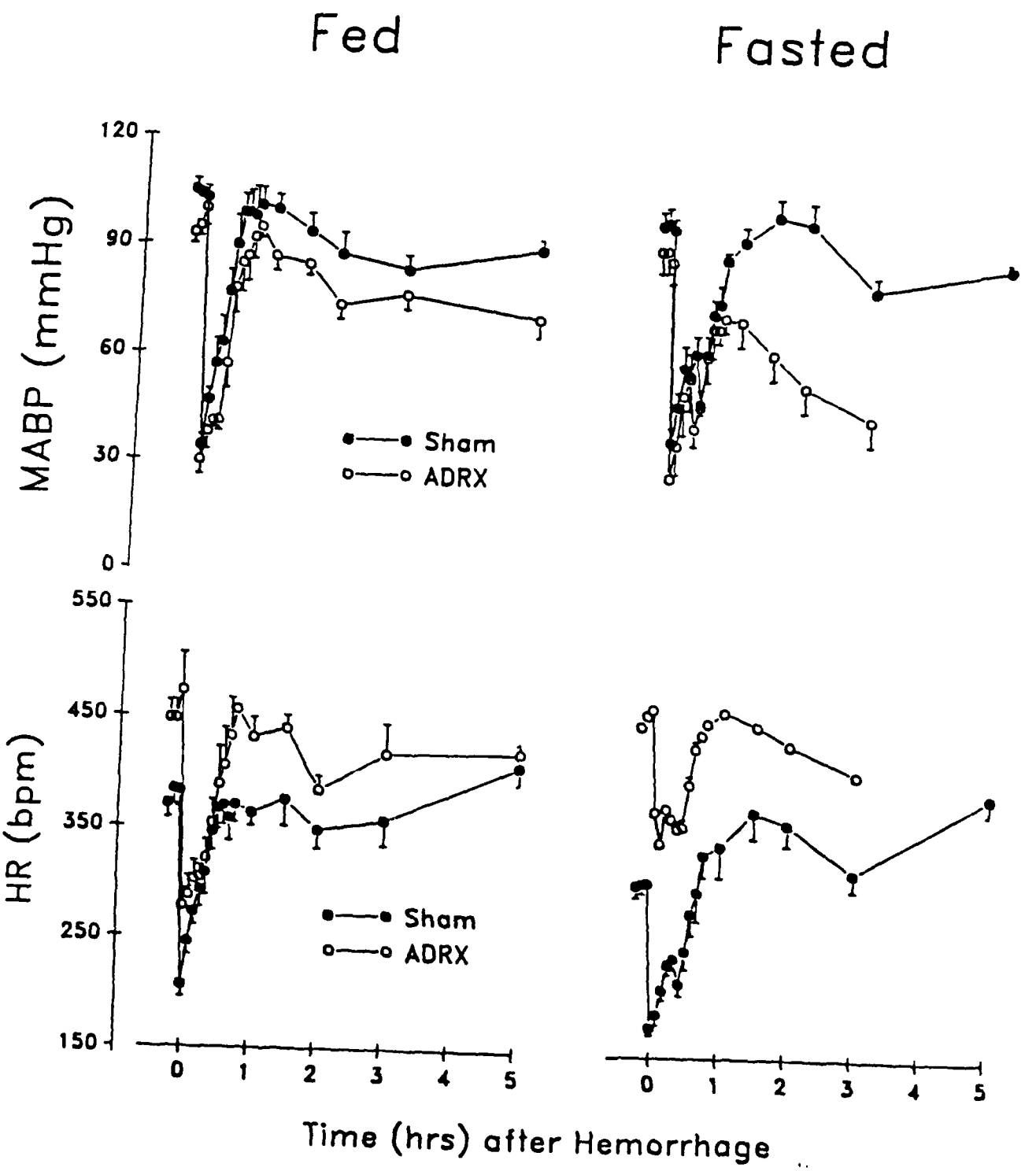

Sham n=8
ADRX n=7
130
100
70
PO_2 (mmHg)
45
35
25
PCO_2 (mmHg)
7.5
7.4
7.3
pH
−1
0
1
2
3
4
5
Hem
Time (hours)

Figure 9


Fed
Fasted
MABP (mmHg)
120
90
60
30
0
Sham
ADRX
HR (bpm)
550
450
350
250
150
0
1
2
3
4
5
Time (hrs) after Hemorrhage

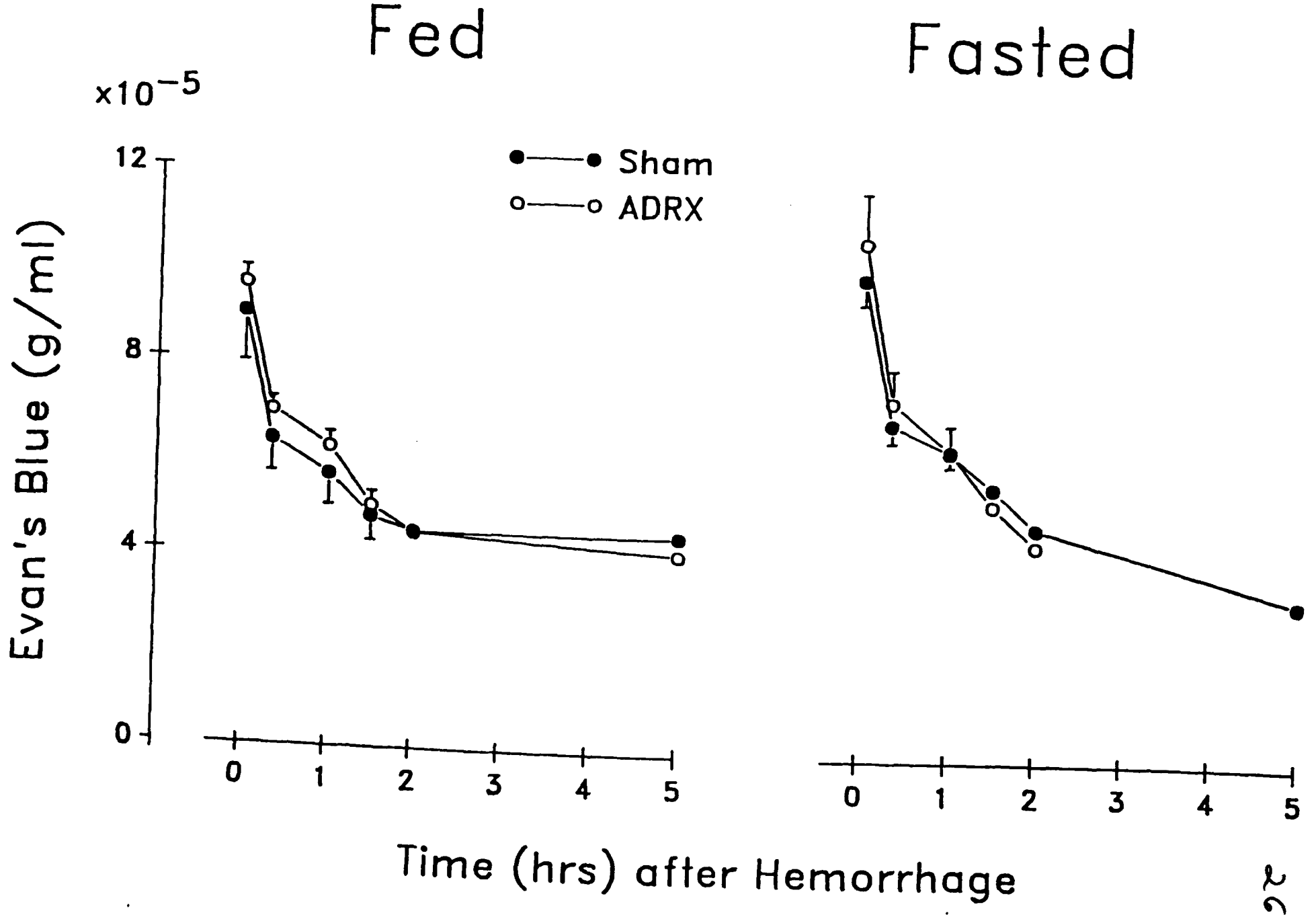

Fed
Fasted
x10⁻⁵
12
8
4
0
Sham
ADRX
Evan's Blue (g/ml)
0
1
2
3
4
5
Time (hrs) after Hemorrhage

Figure 11

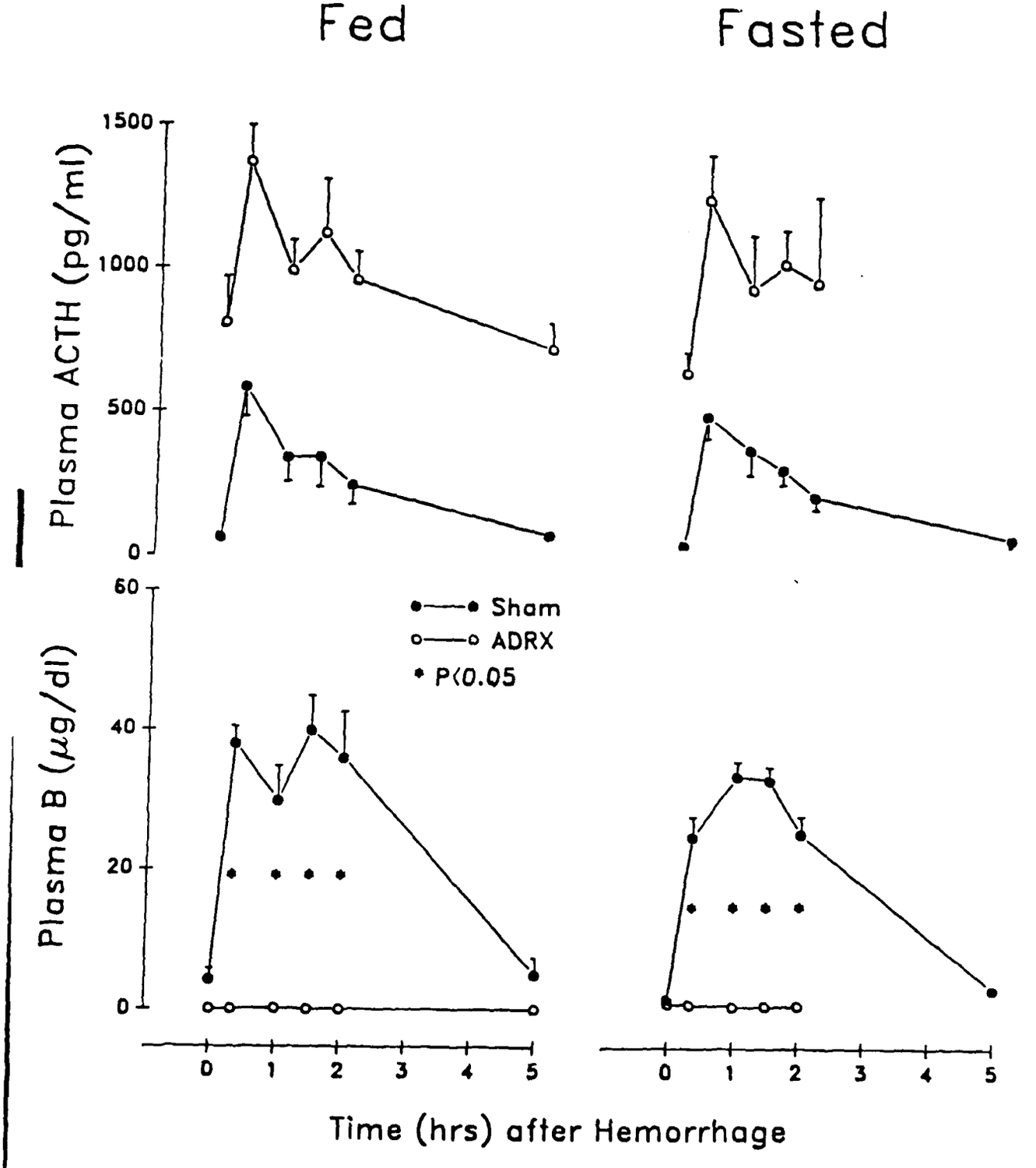
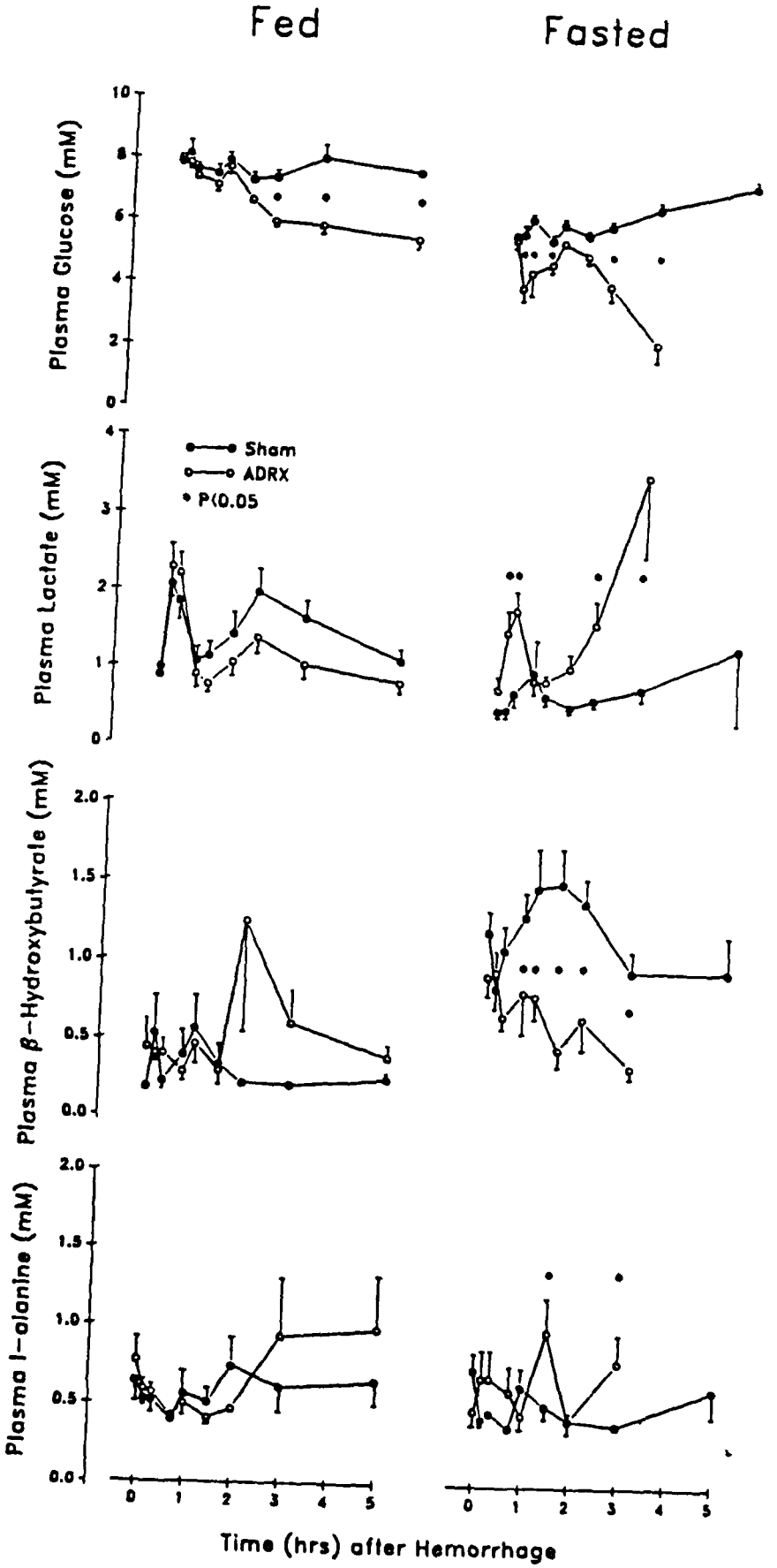

Fed
Fasted
Plasma ACTH (pg/ml)
1500
1000
500
0
Plasma B (μg/dl)
60
40
20
0
Sham
ADRX
* P<0.05
0
1
2
3
4
5
Time (hrs) after Hemorrhage

Figure 12


Fed
Fasted
Plasma Glucose (mM)
Plasma Lactate (mM)
Plasma β-Hydroxybutyrate (mM)
Plasma l-alanine (mM)
Sham
ADRX
* P<0.05
Time (hrs) after Hemorrhage